15 Endocrinology

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INTRODUCTION

Communication between cells and organs is essential for the coordination of physiological processes, including those that adapt animals in time and space to their environment. Classically, the nervous system and the endocrine system were considered complementary for communication in a complex organism. The developments of the last two decades have not only shown that both systems operate in a highly integrated way, but also that they show a substantial overlap with respect to the mechanisms and messengers they employ. Endocrine messengers have been detected in cells, tissues, and organs other than the classical endocrine glands and tissues. For instance, in the vertebrates, including fishes, somatostatins are produced in the islet tissue, the gut, as well as in the brain; in mammals, and probably other vertebrates, arginine vasotocin is not only produced in the brain but also in peripheral organs, including the gonads, and calcitonin gene expression has been established in the nervous system and in pulmonary neuroepithelial cells, in addition to the well-known locations in the C-cells of the thyroid gland or the ultimobranchials. The messengers concerned may function, depending on their site of production, as neurohormones, neurotransmitters, or classical hormones. These and similar discoveries have led to the growing awareness that the boundaries between the nervous and endocrine systems, from an endocrine point of view, are arbitrary. This has resulted in the neuroendocrine concept of regulation. It has also become clear that there is an intricate system of intercellular communication at the tissue level, with mechanisms defined as paracrine, autocrine, or intracrine interactions, that shares an important element with the neuroendocrine system: communication accomplished by chemical messengers that evoke a physiological response by binding to specific receptors (Bern, 1990a).

The new developments have been initiated and facilitated by technical innovations, such as improved separation techniques and more sensitive assay methods, and by the introduction in the neuroendocrinological research of new techniques, such as protein and gene sequencing, immunocytochemistry, and *in situ* hybridization. The latter techniques have led to an explosive growth of the number of known messenger molecules, in particular peptides and proteins. This started in the 1970s and still continues.

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Groups of these molecules appeared to be structurally related and have been classified as peptide or protein families, such as the vasopressin, proopiomelanocortin, somatostatin, neuropeptide-Y, and glucagon families, with members functioning, even in one species, as neurotransmitters, neuromodulators, hormones, and/or paracrine messengers, and occurring in a large variety of animal groups (Sherwood and Parker, 1990). Many peptide hormones long considered typical for the vertebrates have now been found in the invertebrates, and messengers first identified in invertebrates, FMRFamide-like peptides, have now been found in the vertebrate nervous system. Even steroids, catecholamines, and peptides that function as hormones in the metazoa have been identified in bacteria and protozoa, frequently in association with specific binding proteins. This marks the long evolutionary history of the genes coding for these messengers (Scharrer, 1990). Pearse and Pollack (1971) have suggested that a large group of peptide-producing endocrine cells that had APUD-characteristics (the capacity for the uptake and decarboxylation of amine precursors) have a common embryological origin, from the neural crest. This generalization turned out to be too rigorous, however, and an underestimation of the variability of these cells with respect to their embryological origin and biochemical characteristics.

In the vertebrates the peptide and protein messengers are produced in the brain and in the classical endocrine organs, as well as in many other organs including the heart, gut, skin, and the lymphoid system. The lymphoid system most clearly exemplifies the new developments in the field of endocrinology. Its cells produce a variety of peptide and protein messengers that regulate the immune response and in addition modulate the hypothalamo-pituitary adrenal/interrenal axis. In this way they represent essential elements of the integrated stress response. These messengers include cytokines typical for the immune system such as the interleukins, as well as products identical, similar, or related to classical hormones, e.g., prolactin, adrenocorticotropic hormone (ACTH), α-melanophore-stimulating hormone (α-MSH), growth hormone, and thyroid- and follicle-stimulating hormones (Weigent and Blalock, 1987). The first results on fishes have now become available.

Thus, the field of endocrinology can no longer be clearly delineated, and this also applies to the endocrinology of fishes. In this chapter we are dealing mainly with the endocrine system of fishes in the classical way. This is rather a matter of convenience than an approach that is self-evident. The chapter intends, first, to show how the recent developments in the field have changed our view on the endocrine system of fishes and, second, to identify the specific aspects of this system that are related to the aquatic habitat of fishes.

THE MESSENGERS

Hypophyseal Hormones

Like all vertebrates, fishes have a neurohypophysis which is continuous with the floor of the diencephalon, and an adenohypophysis which originates

from non-neural tissues. In most fishes the adenohypophysis is differentiated into a rostral and a proximal pars distalis and a pars intermedia (Figure 1). For a detailed description of the embryology and morphology of the hypophysis in fishes one is referred to Schreibman (1986).

Neurohypophyseal Factors

In the neurohypophysis, where many axons terminate that originate from hypothalamic nuclei, storage and release of neurohormones takes place. There are also direct nervous connections between the neurohypophysis and the adenohypophysis, which are typical for fishes (with the exception of the agnathans). In the nonteleostean fishes the interdigitation of the neurohypophysis is confined to the region of the pars intermedia, a condition related to that of the higher vertebrates. In these fishes a primitive median eminence and associated portal system may be present. In the teleosts an anterior and a caudal part of the neurohypophysis can be distinguished. The caudal part is in contact with the pars intermedia. The anterior part invades the proximal pars distalis of the adenohypophysis, and a median eminence or portal system is absent or, at most, poorly developed.

Part of the hormones of the neurohypophysis are released into the general circulation. Like in other vertebrates, several of these belong to the family of vasopressin-related nonapeptides. Of the ten members of this family identified in the vertebrates, seven have been found in fishes. These are mainly produced in the preoptic nuclei. In agnathans only arginine vasotocin has been demonstrated. In chondrichthyans glumitocin, aspartocin, and valitocin have been

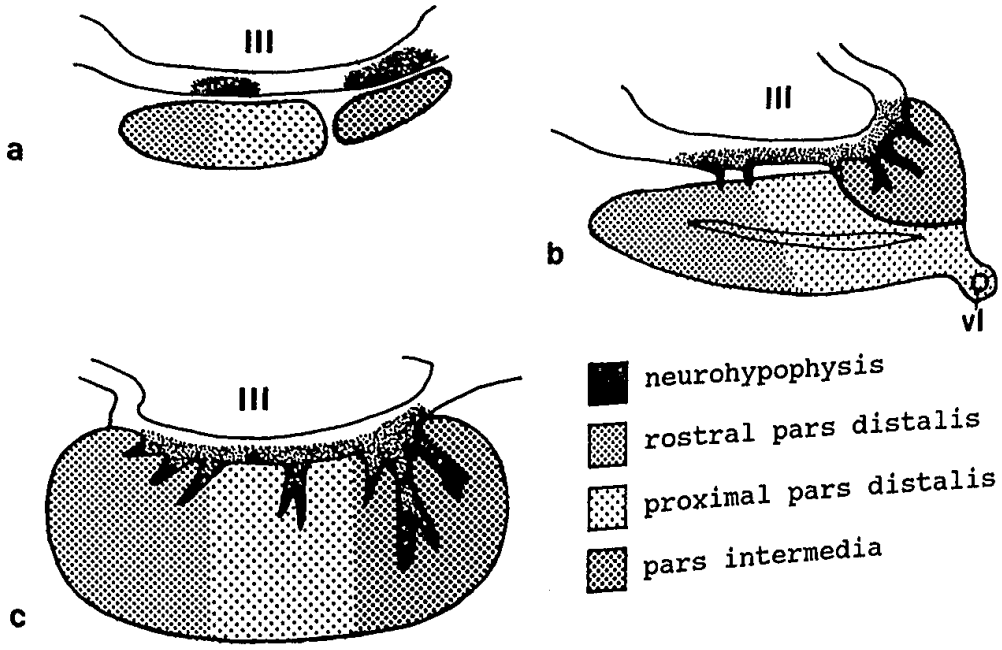

FIGURE 1. Representation of a midsagittal section of the pituitary glands of fishes: (a) agnathan (lamprey; in the hagfishes a pars intermedia is absent); (b) chondrichthyan (shark), vl, ventral lobe; (c) actinopterygian (teleost).

found. Most bony fishes have arginine vasotocin and isotocin, a peptide related to oxytocin, whereas in lungfishes arginine vasotocin, oxytocin, and mesotocin are present (Perks, 1987). The precise function of these peptides has not been clarified. Most of them have effects on blood pressure and, directly or indirectly, on fluid volume control. Diuretic and antidiuretic effects have been reported for arginine vasotocin and isotocin in teleosts. The diuretic actions may be due to the use of nonphysiologically high doses of the hormones (Balment et al., 1993). Transfer of teleosts from fresh water to seawater usually leads to depletion of the arginine vasotocin stores in the neurohypophysis, whereas the reverse occurs after transfer from seawater to lower salinities (Haruta et al., 1991). Plasma levels of this peptide in eels were higher in seawater than in freshwater eels, but this could not be confirmed for flounder and trout in which the higher levels were found in fish from fresh water (Perrott et al., 1991). Administration of arginine vasotocin elicits a spawning reflex. It further stimulates cortisol secretion in the goldfish. This effect may be mediated by ACTH, because arginine vasotocin is colocated with corticotropin-releasing hormone in hypothalamic fibers innervating the ACTH cells in some teleosts (Moons et al., 1991). Arginine vasotocin as well as isotocin promote gonadal testosterone production *in vitro* in rainbow trout testis (Rodriguez and Specker, 1991).

Another group of neuropeptides are released in the neurohypophysis and adjacent areas. These are the factors that regulate the secretion of endocrine cells of the adenohypophysis. Immunoreactivity has been reported for a large variety of peptide hormones. These include growth hormone-, corticotropin-, thyrotropin-, and gonadotropin-releasing hormones, melanophore-concentrating hormone, enkephalin, glucagon-like peptide, FMRFamide-related peptides, somatostatins (in particular somatostatin-14), galanin, neuropeptide Y, bombesin, vasointestinal peptide, and gastrin-like peptides (e.g., Batten et al., 1990; Peter et al., 1990). This list is certainly not complete. Of some of these peptides, two (gonadotropin-releasing hormone) or more (somatostatin) molecular forms have been demonstrated within one species. Many are produced in the preoptic nuclei, but several other hypothalamic nuclei are involved as well. Depending on the species, the peptides may be released into blood vessels and intercellular spaces, and function as hormones, or (with exception of the agnathans), in axons ending on the adenohypophyseal cells. Some of the peptides may also have effects outside the brain and pituitary gland. Melanophore-concentrating hormone, which inhibits α-MSH secretion via fibers that are ending on or nearby the α-MSH-cells in the pars intermedia, is also released in the general circulation and opposes the stimulating action of MSH on the skin melanophores of teleostean and holostean fish. Interestingly, melanophore-concentrating hormone was first isolated from the chum salmon hypophysis 10 years ago, and was subsequently identified in the hypothalamus of other vertebrates, including lampreys, amphibians, and mammals, where it may function mainly as a neurotransmitter and neuromodulator (Baker, 1991).

In addition to peptidergic fibers, serotonergic, noradrenergic, dopaminergic, and GABA-ergic fibers are present, which may end synaptically on, or nearby, the endocrine cells. In the higher vertebrates two or more of these factors may be colocated in the same nerve fibers. This phenomenon does also occur in fishes, e.g., colocation of corticotropin-releasing hormone with arginine vasotocin or isotocin (Fryer, 1989), or with galanin (Batten et al., 1990).

Each adenohypophyseal cell type probably receives a multiple input from the neurohypophysis, as is indicated by immunohistochemical and biochemical observations. For instance, teleost prolactin secretion *in vitro* is stimulated by corticotropin- and thyroid hormone-releasing hormone and by serotonin, and inhibited by somatostatin, urotensin I, vasointestinal peptide, and dopamine (Grau and Helms, 1990). Similar lists can be presented for growth hormone, gonadotropic hormone, and α-MSH. Although the significance of these effects *in vivo* remains to be established for most of these factors, it is clear that the endocrine cells of the pituitary gland are under a complicated multifactorial control. This control may further include paracrine mechanisms that are still poorly understood. This indicates that these cells are centers of neuroendocrine integration.

Adenohypophyseal Factors

The location of the endocrine cell types of the adenohypophysis has been studied in a large number of fish species, originally mainly with antisera raised against mammalian hormones. Later on more and more antisera against fish hormones have become available, with essentially similar results. With the exception of agnathans, fishes show a clear zonation with respect to the location of the endocrine cell types of the adenohypophysis, as is shown for teleosts and elasmobranchs (Figure 1). Glia-like stellate cells have frequently been noticed, e.g., in the rostral pars distalis and the pars intermedia of teleost. Evidence for a regulatory function of these cells, as has been found recently in higher vertebrates, remains to be demonstrated in fishes. Immunocytochemical evidence for the presence of prolactin, somatotropic, corticotropic, thyrotropic, gonadotropic, and melanotropic activity has been reported for chondrichthyan and osteichthyan fishes. For the agnathans corticotropic, thyrotropic, gonadotropic, and melanotropic cells have been reported. Some other typical adenohypophyseal hormones are released in agnathans from the neurohypophysis (see below). Osteichthyans have a unique cell type that is located in the pars intermedia: the PAS-positive or somatolactin cells. The different hormones produced by the adenohypophyseal cells will now be discussed briefly.

The Prolactin Family: Prolactin, Growth Hormone and Somatolactin

The prolactin family includes prolactin, growth hormone, somatolactin, and, in mammals, placental lactogens and proliferins.

Prolactin, the hormone that received its name from its stimulating action on milk production in mammals, has a variety of effects in the higher vertebrates, but no clearly delineated function. In fishes it is essential for hydromineral control in fresh water (Hirano, 1986). Prolactin cells are prominent in the pituitary gland of all fishes except for the agnathans. Prolactin immunoreactivity has further been demonstrated in nerve fibers in the brain. In the agnathans such fibers terminate in the neurohypophyseal region adjacent to the adenohypophysis, and it is possible that from here the hormone may enter the systemic circulation (Schreibman, 1986).

Removal of the pituitary gland of teleost fishes in fresh water leads to losses of body electrolytes that may be lethal. These losses can be reduced or completely redressed by administration of mammalian or teleost prolactin preparations. The major function of the hormone most likely is the maintenance of the permeability to water and ions of epithelia of the skin, in particular the gills, the intestine, and the renal tubules including the bladder, at the low level that is required for surviving of fishes in water with a low salinity. The substantial ionic and osmotic gradients between the body fluids and the water promote passive losses of ions and the osmotic uptake of water, mainly across the integument. For maintaining hydromineral equilibrium, the ion losses have to be kept at a level that can be compensated for by active ion uptake. The low permeability of the renal tubular epithelia is essential for the production of large volumes of hyposmotic urine, to compensate for the osmotic water inflow. These actions of prolactin are of particular importance during migration of euryhaline fishes from seawater to fresh water (Hirano, 1986; Wendelaar Bonga and Pang, 1991).

In some teleosts, prolactin has hypercalcemic effects, but these may be related to the general function in hydromineral control. The hormone does not seem to have a specific function in calcium homeostasis (Wendelaar Bonga and Pang, 1991). Many pollutants, as well as stressors such as handling, induce hydromineral imbalance in fishes, and this may evoke a prominent response of the prolactin cells. This has been shown for several freshwater teleosts, with the exception of the salmonids. In this group the role of prolactin in hydromineral control is unclear. The function of prolactin in seawater fishes is unknown. The circulating hormone levels are much lower than in freshwater fishes. Apart from the control of the hydromineral balance in teleosts, prolactin has been implicated in the control of gonadal steroidogenesis (Singh et al., 1988) and of parental fanning and in lipid metabolism (Wendelaar Bonga and Pang, 1989). With cDNA cloning, two different forms of prolactin have been identified in several teleost species. Notable functional differences have so far not been demonstrated (Specker et al., 1985; Swennen et al., 1991).

Growth hormone, like prolactin, is present in the adenohypophysis of all fishes except for the agnathans, where growth hormone-immunoreactivity has been demonstrated in fibers in the neurohypophysis. Two structural variants of growth hormone occur in the teleosts examined. The actions of the hormone seem to be mediated, like in the higher vertebrates, by insulin-like growth factors

(IGF), which are members of the insulin family. In mammals two forms predominate, IGF-I and IGF-II. Data on fishes are scarce. Some IGF-I-like immunoreactivity has been demonstrated in the plasma of teleost and elasmobranch fishes (Daughady et al., 1985), including salmon, in which a rise was reported during adaptation to seawater (smoltification; Lindahl et al., 1985). Indications for the production of IGF have been demonstrated recently in some teleosts, for instance in salmonid liver, gills, and kidney (Sakamoto et al., 1993). Growth hormone stimulates body growth and the peripheral conversion of T4 to T3, at least in eels and salmonids (see below). In addition, the hormone promotes the development of the capacity for hypo-osmoregulation during smoltification, probably in synergism with cortisol (Madsen, 1990). It stimulates chloride cell development and Na^+/K^+-ATPase activity in the gills. Plasma growth hormone concentration and turnover increase during seawater adaptation in salmonids (Sakamoto et al., 1991). Whether growth hormone stimulates seawater adaptation in other euryhaline fish is still unclear. Growth hormone also has been implicated in the inhibitory control of antifreeze protein production in winter flounder (Idler et al., 1989) and in gonadal steroid synthesis (Van der Kraak et al., 1990).

Somatolactin — the predicted amino acid sequence of the hormone of the somatolactin cells of the pars intermedia cells has been reported recently for flounder (Ono et al., 1990). This hormone has structural homology with prolactin and growth hormone, and this resulted in the name somatolactin. Like growth hormone, it is glycosylated. Its function is unclear. The somatolactin cells of teleosts (Figure 2) respond to a variety of stimuli, such as the calcium concentration, pH, or salinity of the water, and change in background color (Wendelaar Bonga and Pang, 1989). Plasma somatolactin levels increased markedly during gonadal growth in coho salmon, and somatolactin stimulated steroidogenesis of gonadal tissue of coho salmon *in vitro* (Planas et al., 1992).

The POMC-Gene Derived Peptides: ACTH and MSH Cells

The ACTH and MSH cells, which have been identified in the pituitary gland of all groups of fishes (Schreibman, 1986), synthesize and release peptides which originate from a common precursor molecule, proopiomelanocortin (POMC). This precursor molecule, which is also found, for example, in mammalian lymphocytes (Weigent and Blalock, 1987) and in the brains of many vertebrates, including fishes (Olivereau and Olivereau, 1990), is processed in a way that is specific for each cell type. In the ACTH cells of the vertebrate pituitary gland, this results in an N-terminal peptide, in addition to ACTH and lipotropin (β-LPH) as end products. In the MSH cells these peptides are processed further: ACTH yields α-MSH and corticotropin-like intermediate lobe peptide, while β-LPH is cleaved to give β-endorphin and γ-LPH. The latter may be processed further to β-MSH, and the N-terminal fragment to γ-MSH (Figure 3). In general, fishes (there are data from lungfish, elasmobranchs, and actinopterygians) seem to follow this

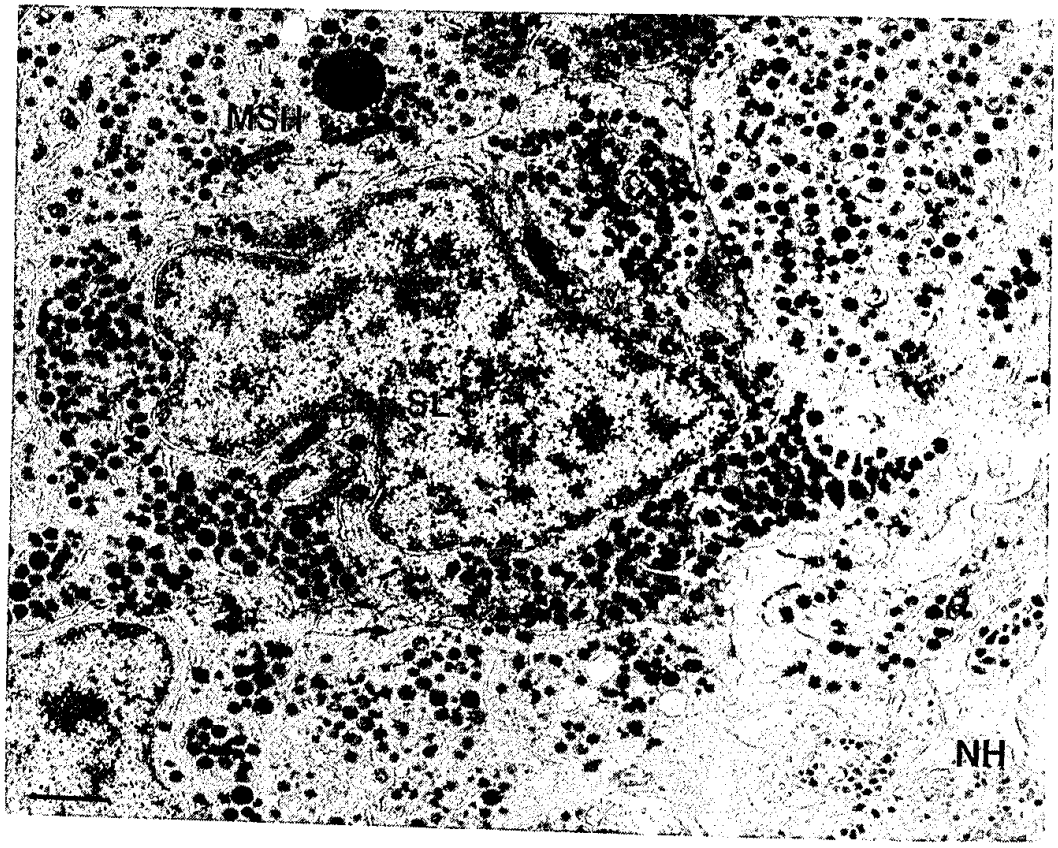

FIGURE 2. Electron micrograph of the pars intermedia of the pituitary gland of goldfish; somatolactin cell (SL) surrounded by melanotropic cells (MSH); NH, axon terminals of the neurohypophysis; bar represents 1 μm. (From Wendelaar Bonga et al., *Cell Tissue Res.*, 243, 609, 1986. With permission.)

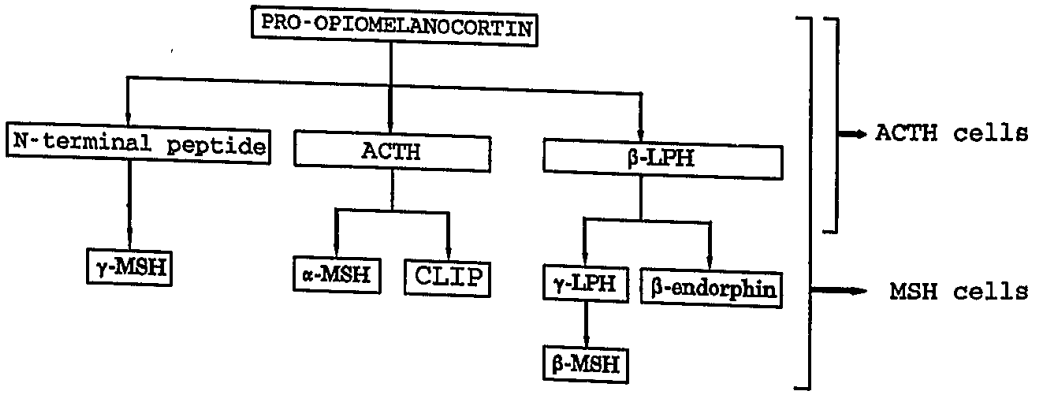

FIGURE 3. A generalized diagram of the processing of proopiomelanocortin (POMC) in ACTH and MSH cells of the vertebrates; ACTH, adrenocorticotropic hormone; LPH, lipotropin; MSH, melanophore-stimulating hormone; CLIP, corticotropin-like intermediate lobe peptide.

or a similar pattern, although γ-MSH has so far only been demonstrated in a dogfish. Two separate POMC molecules encoded by two different genes are present in chum salmon (Kawauchi, 1988). Indications for different forms of ACTH with corticotropic activity were obtained in goldfish (Fryer, 1989).

ACTH probably is the major secretagogue regulating the synthesis and release of corticosteroids in fishes, as it is in the higher vertebrates. The function of the N-terminal peptide is not known. The name MSH was derived from its first known function, i.e., the dispersion of melanin in skin melanophores in amphibians, but its function in many other vertebrate groups, including fishes, is unclear. It has been implicated in melanogenesis and background adaptation of the skin color in some teleosts (Van Eys and Peters, 1981), but in many other fishes no effects on skin pigmentation could be demonstrated. Three forms of α-MSH have been identified in carp, goldfish, and tilapia: des-acetyl, monoacetyl-, and diacetyl-α-MSH. All forms have melanotropic activity *in vitro*. Recently, in mammals, corticotropic activity has been ascribed to α-MSH, and this has also been found in teleost fishes, with di-acetyl—MSH as the most active form (Lamers et al., 1992). Balm et al. (1993) recently reported that treatment with a bacterial endotoxin inhibited α-MSH secretion *in vitro*, and that this effect may be mediated by an interleukin-1-like factor. Bayne and Levy (1991) demonstrated that ACTH stimulates the respiratory burst activity of phagocytes in trout. These data indicate for fishes a type of interaction between the hypothalamo-pituitary-interrenal axis and the immune system comparable to that established in mammals (Weigent and Blalock, 1987).

The Gonadotropin Family: Gonadotropins and Thyrotropin

This family consists of glycoproteins, each consisting of two different subunits (α and β). Within a species the hormones have identical α-subunits, while the β-subunit is hormone specific. The gonadotropins will be discussed in the chapter on reproduction. Thyroid-stimulating hormone is an important stimulator of thyroid function in fishes as it is in higher vertebrates. It has received little attention.

Urotensins

Jawed fishes are unique in having a neuroendocrine system almost at the end of the spinal cord: the caudal neurosecretory system. Typically, it is a ventral swelling of the cord, consisting of axon terminals forming a neurohemal organ, structurally similar to the neurohypophysis. The terminals originate from large neurons, the Dahlgren cells, that are distributed over the caudal part of the spinal cord. In the agnathans, neither a caudal neurosecretory system nor Dahlgren cells have been found. In chondrichthyans, Dahlgren cells are present, but their terminals do not form a swelling and are distributed along the periphery of the caudal spinal cord. This situation is also typical for many osteichthyans. Most teleosts have a well-developed caudal neurosecretory system: the urophysis. Two hormones predominate in the urophysis of teleosts: urotensin I and II. Urotensin I has structural homology to corticotropin-releasing hormone, and urotensin II to the somatostatins. There are subpopulations

of Dahlgren cells producing urotensin I or urotensin II, or both. Immunoreactivity to these peptides has further been demonstrated in the brain, where the urotensin-like peptides may act as neurotransmitters and as hypophysiotropic factors (Bern, 1990b). Both urotensins have myotropic actions and are weakly vasoconstricting, and both have osmoregulatory effects, in particular in seawater. The effects may be partly indirect. Urotensin I stimulates interrenal steroid synthesis. Urotensin II stimulates the contraction of the teleost heart, urinary bladder, intestine, and gonadal ducts, and promotes the intestinal uptake of ions in seawater (Bern, 1990b). It further appeared to be hypoglycemic and enhanced lipogenesis in coho salmon (Sheridan et al., 1987). The release of both hormones seems to be inhibited by transfer of seawater fish to lower salinities, and stimulated by the reverse transfer (Larson and Madani, 1991). Removal of the urophysis and urotensin-producing cell bodies of the spinal cord in goldfish resulted in an increase in hypothalamic urotensin-I-like activity and in increased release of pituitary ACTH and cortisol (Woo et al., 1985). Administration of urotensin-I increased plasma cortisol in flounder (Arnold-Reed and Balment, 1989). These results indicate a complex interaction of the urophysis with the hypothalamo-pituitary-interrenal axis.

Thyroid Hormones

The functional unit of the thyroid tissue of the vertebrates is the epithelial follicle. In amphibians and higher vertebrates these follicles form compact or multilobular, single or paired glands. Contrastingly, in fishes the thyroid tissue displays a large variety of shapes. In agnathans and teleosts the follicles are distributed in the connective tissue ventral to the pharynx. In some species the follicles may be found outside this area, mostly in the head kidney. In only a few teleosts, the follicles are concentrated in one or more compact lobes or glands. A single gland, located ventromedially to the pharynx, is found in chondrichthyans, lungfishes, and crossopterygians. In lampreys, the thyroid develops from the endostyle, a ciliated pharyngeal groove present in the larva. In all jawed fishes the thyroid follicles arise from the ventral wall of the pharynx, posterior to the first pair of pharyngeal pouches. The structure of the follicles is very similar from agnathans to mammals (Dent, 1986).

The gland cells actively concentrate iodine from the blood and, after oxidation, this is bound to the tyrosyl groups of a protein produced by these cells, thyroglobulin. This protein is synthesized in the gland cells and is secreted into, and temporarily stored in, the follicular lumen, where iodination takes place. When the cells are stimulated by TSH, the iodinated thyroglobulins are taken up from the tissues by endocytosis, and partly destroyed in lysosomes. This leads to the release of the iodothyrosines, mainly tetra-iodothyrosine (thyroxin or T4) which is secreted into the blood. In the target tissues, such as liver and gills, it is deiodinated to tri-iodothyrosine (T3), the biologically more active form, under the control of hormones, including growth hormone and testosterone. Cortisol may have a reverse effect (Dent, 1986; Eales and McLatchy, 1989).

Thyroid hormones are important for the control of metabolism, growth, and development, and osmoregulation in fish as well as in higher vertebrates. They frequently act in synergism with growth hormone or cortisol. Thyroid hormones stimulate metamorphosis of flounders, comparable to their effect on amphibian metamorphosis. Increased activity of the pituitary cells producing thyroid-stimulating hormone and a peak of plasma T4 coincident with a cortisol peak, has been observed during the climax of metamorphosis (Grace de Jesus et al., 1991). Thyroid hormones of maternal origin have been identified in the yolk of unfertilized fish eggs, and these hormones may be of importance for the regulation of early development (Brown and Bern, 1989). Another important function of thyroid hormones is the triggering of migratory behavior and the control of osmoregulatory adaptation to seawater in salmonids and possibly other migratory fishes. High surges of T4 and T3 have been reported during the parr-smolt transformation in fresh water, days or weeks before a peak in branchial Na^+/K^+-ATPase activity, an indicator of seawater preadaptation, is displayed. A link has been demonstrated between the T4 peaks and the lunar cycle during this preadaptive period (Grau, 1987). Freshwater to seawater transfer of trout involves an increase of T4 and T3 metabolism, and of deiodination of T4 to T3 (de Luze et al., 1987). This has also been shown by the same authors for eels, although the data on the relationship between plasma T3 and T4 levels and seawater adaptation in nonsalmonid euryhaline species are less consistent than in salmonids.

Interrenal Hormones

The homologue in fishes of the adrenal gland of the higher vertebrates is the interrenal tissue. In adrenal glands, the medullary tissue is of neural crest origin and produces catecholamines. It is surrounded by a cortex of steroid-producing tissue, which originates from the mesoderm. The interrenal tissue of fishes shows a large heterogeneity with respect to the medullary and cortical homology, and no true medulla or cortex can be distinguished. The term interrenal is usually, although not consistently, restricted to the steroid-producing cells, whereas the catecholamine-producing elements are called chromaffin cells. In the agnathans, the interrenal cells are distributed in groups in the pronephric region. In the chondrichthyans one or more large groups of interrenal cells are spatially separated from small groups of chromaffin cells, which are located on the surface of the kidneys. In most teleost fishes (Figure 4) both cell types are intermingled and located around the posterior cardinal veins in the pronephric part of the kidneys, the head kidneys (Chester Jones and Phillips, 1986).

The Interrenal Steroid-Producing Cells

Low plasma levels of cortisol, corticosterone, and deoxycortisol have been demonstrated in agnathans. The major corticosteroid produced in most gnathostomes is cortisol. In elasmobranchs it is 1α-hydroxycorticosterone. Only traces of other steroids such as corticosterone (most groups) or aldosterone

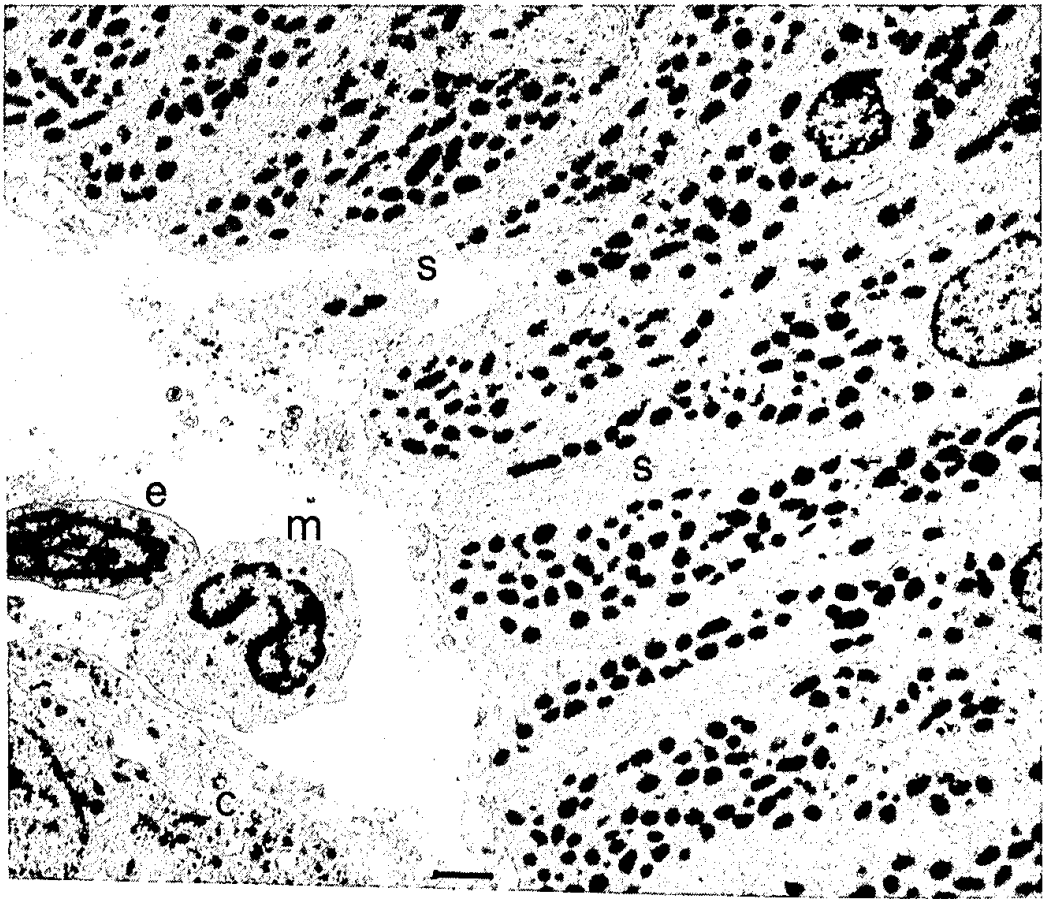

FIGURE 4. Electron micrograph of the wall of a branch of the posterior cardinal veins, showing steroid-producing interrenal cells (s) and chromaffin cells (c) of the teleost *Oreochromis mossambicus*; e, erythrocyte; m, monocyte-like leukocyte; bar represents 1 μm.

(lungfishes) have been detected (Henderson and Kime, 1987). Cortisol is involved in energy metabolism, ion regulation, and in the responses to stressors.

Cortisol is hyperglycemic by stimulating glycolysis and gluconeogenesis from protein and lipid sources. It activates key enzymes for hepatic intermediary metabolism, as was shown in chondrichthyans and teleosts (Vijayan et al., 1991). The ionic regulatory function of corticosteroids is very important because the osmoregulatory problems faced by most fishes are substantial. Freshwater fishes are hyperregulators, and thus face hyperhydration and diffusive ion losses. Osmotic and ionic homeostasis is maintained by the active uptake of ions through the gut and, in particular, across the gills. The marine jawless fishes are osmoconformers. The composition of the main ions of their blood plasma reflects that of seawater. In the marine chondrichthyans the blood plasma is slightly hyperosmotic because of a high content of urea. The relatively low salt levels of the plasma are maintained by the secretion of sodium and chloride by the rectal salt gland. Seawater teleost fishes are hyporegulators; they maintain their plasma osmotic value at a level about one third that of

seawater, i.e., only slightly higher than that of freshwater fishes. The resulting osmotic water losses are compensated by drinking seawater. The water uptake is driven by a $Na^+/K^+/Cl^-$ cotransporter in the intestinal epithelium. The high uptake of monovalent ions is compensated by the excretion of these ions via the chloride cells in the gills (see Chapter 11, this volume). The control of the ion-regulatory processes is dependent to a high degree on corticosteroids and this most likely holds for all fishes, even though data on fishes other than teleosts are limited (Henderson and Kime, 1987). Corticosteroid injections decrease salt losses in lampreys when in fresh water, and stimulate salt excretion by the rectal glands in chondrichthyans. In both freshwater and seawater teleost fishes ionic regulation is controlled by cortisol, which is known to stimulate the proliferation of chloride cells and to promote the Na^+/K^+-ATPase activity of these cells, the driving force for monovalent ion transport in both fresh water and seawater. Cortisol secretion is stimulated transiently during migration or transfer of euryhaline fishes from fresh water to seawater, and this has led to the designation of cortisol as a seawater adaptation hormone. This is no longer tenable because cortisol secretion is also enhanced during seawater-to-freshwater transfer: it is indispensable as a gluco- and mineralocorticoid in freshwater as well as seawater fishes (Mayer-Gostan et al., 1987; Laurent and Perry, 1990).

Cortisol secretion is stimulated in response to many stressful stimuli, varying from handling, confinement, and other forms of disturbance, to toxicants and water acidification. This has led to the recognition of cortisol as an important stress hormone in freshwater as well as seawater fishes, like it is in the terrestrial vertebrates (Schreck et al., 1989). This function of cortisol can be related directly to the circumstance that stressful stimuli induce hydromineral disturbances and require the mobilization of energy reserves (Bonga and Lock, 1992). Cortisol probably mediates the immune suppression and the inhibition of growth and reproduction that may occur during prolonged and severe stress (Carragher et al., 1989). Enhanced cortisol secretion is often associated with gonadal maturation (Schreck et al., 1989). A remarkable and still not fully understood phenomenon is the hyperplasia of the interrenal cells during sexual maturation, for instance in some salmonid species during and after migration to their spawning grounds, which may end in large-scale post-spawning mortality (Henderson and Kime, 1987).

The many actions of cortisol are reflected by the complexity of the mechanisms that control its secretion. In addition to ACTH, the major secretagogue, and α-MSH (see above), hormones such as growth hormone, thyroxin, angiotensin II, arginine vasotocin, and catecholamines may have corticotropic activities (Schreck et al., 1989). Factors such as the N-terminal peptide of POMC (see above) and factors produced by the immune system may modulate cortisol secretion directly or by affecting other regulatory components of the hypothalamo-pituitary-interrenal axis (Balm et al., 1993).

The Catecholamines

The chromaffin cells synthesize epinephrine and norepinephrine as the main end products. In addition, important amounts of dopamine may be produced. The significance of the dopamine release is unknown. In teleosts, epinephrine and norepinephrine are produced in specific cell types. Distinct dopamine cells seem to be absent. In eels the colocalization of catecholamines with peptides such as methionine enkephalin has been demonstrated. In fishes, the bulk of the circulating catecholamines is released from chromaffin cells associated with the cardiovascular system, in particular those located in the head kidney region. This contrasts with the situation in mammals, where there is also a substantial "overflow" from sympathetic nerves to the blood (Epple et al., 1989). Catecholamines in fishes have effects on the blood circulation, respiration, and energy balance, like in other vertebrates. Hyperglycemic effects of epinephrine and norepinephrine have been reported for all major groups of fishes, although the significance of the data has been doubted because of the high doses used in many experiments (Epple et al., 1989). In teleosts, epinephrine and norepinephrine stimulate both glycogenolysis and gluconeogenesis, effects mediated by β-adrenoceptors. The relative potency of both catecholamines differs between species (Danulat and Mommsen, 1990). Typical for fishes is the important role of catecholamines, in particular epinephrine, for the control of the vasculature of the gills (Mayer-Gostan et al., 1987). This vasculature has a higher sensitivity to catecholamines than the systemic vessels. Typically, administration of epinephrine produces a biphasic response: an initial vasoconstriction that can be blocked by α-adrenoreceptor antagonists is followed by a more prolonged vasodilation that can be inhibited by β-antagonists (Wahlqvist, 1981). The vasodilating effect that has been produced, for example, in agnathans, chondrichthyans, and teleosts promotes perfusion of the gill lamellae and thus stimulates oxygen uptake. The oxygen supply of the body tissues is further promoted by the stimulatory effects on ventilatory movements and by direct effects on the erythrocyte volume and intracellular pH. Upon β-adrenergic activation of the Na^+/H^+ antiporter in the erythrocyte membrane, which leads to a rise in intracellular pH, the affinity and capacity of oxygen binding to hemoglobin is enhanced (Aota et al., 1990). The above effects are of primary importance for the rapid mobilization of energy during exposure to a variety of stressors. The catecholamines are important stress hormones in fish, like in the higher vertebrates (Mazeaud and Mazeaud, 1981; Epple et al., 1989).

Several effects of catecholamines on osmoregulation have been reported. They promote ion exchanges across the gills, probably as an indirect effect of their stimulatory action on gill perfusion, which increases the area of the gill lamellae that is in direct contact with the water. In seawater teleost fishes chloride secretion by the chloride cells can be inhibited by α-, and stimulated by β-adrenergic mechanisms (Mayer-Gostan et al., 1987).

In teleost fishes the release of catecholamines is under the control of cholinergic nerve fibers and of humoral factors. In this respect, the teleosts are intermediate between the cyclostomes, which are lacking nervous innervation of the interrenal steroid-producing and chromaffin cells, and mammals, in which the adrenomedullary secretion of catecholamines is fully dependent on cholinergic innervation. Hypoxia and blood acidosis strongly increase ventilatory movements, and this effect is mediated by catecholamines. High P_{CO2} and low blood pH are important stimulants of catecholamine secretion (Aota et al., 1990). Other stimulants seem to be the hormones themselves: at least in eels an increase of the blood level of one of the three catecholamines stimulates the release of the other two. This effect was not influenced by removal of the brain and spinal cord destruction (Epple et al., 1989). The blood-borne factors may control the catecholamine secretion under normal conditions, whereas the cholinergic innervation may mediate the rapid responses of these cells to stressors: sectioning of the nervous supply to the chromaffin cells resulted in a decreased ability to respond to stressors (Wahlqvist and Nilsson, 1980).

Natriuretic Peptides

A new and exciting development in vertebrate endocrinology is the discovery of the natriuretic peptide family, a group of closely related peptides of about 3 kDa. They were detected in the early 1980s, and are produced in several tissues, in particular the brain, where they function as neurotransmitters, and in cardiac tissue. The cardiac peptides, synthesized and released in atrial as well as (in the lower vertebrates) ventricular cells, function as hormones. The amino acid sequences of the peptides from the three sources may differ slightly, and they are called A-type natriuretic peptide (first found in the atrium) B-type and C-type natriuretic peptides (first identified in the brain) and V-type natriuretic peptide. The latter is a novel type, isolated from the eel ventricle (Takei et al., 1991). In mammals the natriuretic peptides have potent natriuretic, diuretic, and vasodilatory actions, which result in a decrease of blood pressure. They act directly, by decreasing tubular sodium resorption and increasing the glomerular filtration rate in the kidneys and by dilating contracted arteries, as well as indirectly, by inhibiting hormones such as aldosterone, vasopressin, or angiotensin. Synthesis and release of the cardiac peptides are controlled by factors connected with an increase in blood volume such as atrial and ventricular stretch and increased plasma Na^+ or osmolarity. In addition, various hormones including glucocorticoids, thyroid hormone, angiotensin II, and endothelins (the vasoconstrictive peptides produced by the vascular endothelium) may also control secretion (Evans, 1990, in press).

The presence of natriuretic peptides in fish has been demonstrated in brains, cardiac tissues and blood plasma of agnathans, chondrichthyans, and teleosts. The available literature has recently been reviewed extensively by Evans (1990; in press) and references for the data mentioned in the following summary can be found in these reviews. Unlike mammals, in fish the ventricular production

seems to be higher than the contribution of the atria. Most data have been obtained by immunocytochemistry and radioimmunoassays with mammalian antisera. Only recently, A-type and C-type natriuretic peptides of two teleosts and two sharks have been sequenced. The first results indicate that, because of amino acid sequence differences, mammalian antisera underestimate the actual natriuretic peptide content of teleost tissues and body fluids.

The intestine, gills, kidneys, and cardiovascular system have been identified as the main targets in fish. In the intestine of seawater flounder, mammalian atrial natriuretic peptide inhibits the active uptake of Na^+ and Cl^-, the driving force for intestinal water uptake. As this water uptake is essential to offset the dehydration of seawater teleosts, this effect seems to counteract the regulation of water balance in seawater fish (O'Grady et al., 1985). Contrastingly, the promotion of salt secretion in seawater fish points to a function in ion regulation that is essential for survival in seawater; the Cl^- extrusion by gills of seawater killifish was stimulated (Scheide and Zadunaisky, 1988), like the salt secretion by the shark rectal gland. The last observation has been confirmed in a shark, with extracts from atrial and ventricular shark tissues. That atrial natriuretic peptides are more important in seawater than in freshwater fish is indicated by the rise of plasma atrial natriuretic peptide levels when euryhaline freshwater fish are exposed to higher salinities, and the reduction of these levels when marine teleosts are acclimated to dilute seawater (Evans et al., 1989, Evans, in press).

In the kidneys, high doses of mammalian atrial natriuretic peptides produce natriuresis and a slight diuresis. Urine flow was reduced in a shark, an effect mediated by the glomerular filtration rate. Antidiuretic activity has further been shown with homologous atrial natriuretic peptide and ventricular natriuretic peptide in freshwater eel. Drinking was reduced at low doses of atrial natriuretic peptide in both freshwater and seawater eels (Takei and Balment, 1993).

Mammalian atrial natriuretic peptides have no effect or produce tachycardia when tested on fish hearts. Mammalian as well as homologous atrial natriuretic peptides induce hypotension in eel and shark. In general, preconstricted teleost vessels *in vitro* respond with relaxation to mammalian or fish atrial natriuretic peptides (Evans, in press). An interesting observation is that the potency of eel and killifish was less than that of rat atrial natriuretic peptide in causing relaxation of aortic rings of toadfish (Evans et al., 1989). Because fish natriuretic peptides have a much higher potency in homologous assays, this indicates substantial interspecies differences between fish. Recently it was reported that human atrial natriuretic peptide increases plasma cortisol levels in seawater flounder, and stimulates cortisol secretion by interrenal tissue *in vitro*. The latter effect could only be produced in tissue from seawater-acclimated fish, and not freshwater fish, and has recently been confirmed with homologous atrial natriuretic peptide in eels for cortisol secretion *in vivo* (Takei and Balment, 1993).

In conclusion, the function of natriuretic peptides in fishes seems more connected with salt regulation than volume regulation and this function seems more important in seawater fishes than in freshwater fishes (Evans, in press). The data are limited, however.

Calcitonin

Calcitonin is produced in the ultimobranchial glands. These occur in all vertebrates except the agnathans, which are lacking calcitonin, and the mammals, where the calcitonin-producing cells are distributed in the thyroid gland. In fishes the gland is unpaired and located in the connective tissue sheets around the heart. The gland consists of endocrine cells, originating from the neural crest, and of nonglandular cells which show structural resemblance to the stellate cells of the pituitary gland. The function of calcitonin in fishes has not been fully clarified. In mammals and birds calcitonin has some hypocalcemic potency, particularly in young animals. The hormone redresses the hypercalcemia resulting from the episodic intestinal calcium uptake after a meal, by stimulating calcium deposition in the skeleton. It further protects the skeleton against excessive demineralization during pregnancy and lactation, by inhibiting osteoclastic activity and osteocytic osteolysis (Wendelaar Bonga and Pang, 1991).

Calcitonins from sharks and some teleosts have been isolated and sequenced and are very potent in mammalian assay systems. Salmon calcitonin is of therapeutic value for man and is used as an analgesic and for treatment of osteoporosis and other bone diseases. However, calcitonin has no clear hypocalcemic potency in fishes. It promotes bone formation by osteoblasts, and this action may be the main function of calcitonin in fishes (Wendelaar Bonga and Lammers, 1982). The bone of fishes is either cellular, with limited numbers of encapsulated osteocytes, or acellular, with osteoblasts and osteocytes limited to the periphery of the bone elements. Osteoclasts have been described, but they are probably scarce in most fishes. Effects of calcitonin on these cells have not been reported.

Calcitonin secretion is stimulated during gonadal maturation in female fishes. This process involves the production in the liver of calcium-containing phospholipoproteins (vitellogenins) and the subsequent transport of these proteins to the ovaries. Whether the high calcitonin secretion during ovarian development, which is under the control of estrogens, is connected with the protection of the skeleton against excessive demineralization, like in mammals, is not known. Demineralization of both cellular and acellular bone is possible and has been demonstrated, for instance, by experimental induction of gonadal maturation by high doses of estrogens. A specific role for calcitonin in gonadal maturation has also been suggested (Björnsson et al., 1989), but direct evidence is lacking. The interesting possibility has been presented that calcitonin, like other hormones that show peaks in plasma levels during ovarian maturation (see

above), penetrates the eggs and becomes involved in early development of the embryos (Brown and Bern, 1989; Bern, 1990a). The factors controlling calcitonin secretion, with exception of estrogens, have not been identified in fishes.

Immunoreactivity to calcitonin — and to calcitonin gene-related peptide, originating from the calcitonin gene by alternative RNA splicing — has been found in the nervous system, including the neurohypophysis, of different classes of fishes. The peptides may function as neurotransmitters and neuromodulators rather than hormones in these areas (Kaneko et al., 1989).

Stanniocalcin

The corpuscles of Stannius are typical for holosteans and teleosts. In the last 10 years it has become clear that these glands produce a hormone that is unique for these osteichthyan groups and that prevents hypercalcemia. The corpuscles are usually paired and located in or on the kidneys. In holosteans and some teleosts tens of small corpuscles may be present. They arise from the nephric ducts during embryonic development. Surgical removal of the Stannius corpuscles (stanniectomy) causes a pronounced, up to threefold increase of the plasma calcium concentration. This is accounted for by an increase in the protein-bound and the ionic calcium fraction of the blood plasma. This rise takes place in both freshwater and seawater fishes, and is correlated with the water calcium concentration. This indicates that stanniectomy leads to an increase of the inflow of calcium from the water, and this has indeed been demonstrated. The rise of plasma calcium in stanniectomized fish can be redressed by injection of homogenates of the glands (Wendelaar Bonga and Pang, 1991). The active principle, originally called hypocalcin or teleocalcin, has been identified and sequenced in recent years and is now named stanniocalcin (STC). It is a glycoprotein without clear structural homology with other known hormones (Butkus et al., 1987; Wagner and Friesen, 1989). The Stannius corpuscles of many species contain two cell types, one of which produces stanniocalcin; the function of the second cell type is unclear (Figure 5).

Fishes obtain most of their calcium from the water, unlike terrestrial vertebrates, for which food is the only source. In freshwater teleosts the calcium is transported by the chloride cells in the gills. Both in fresh water and seawater, calcium ions enter these cells passively, down the electrochemical gradient, via calcium channels in the apical cell membrane. Subsequently these ions are actively extruded across the basolateral cell membrane of these cells into the blood. This extrusion, against a high electrochemical gradient, is facilitated by ATP-dependent calcium pumps. Stanniocalcin controls this process, probably by acting as a calcium-channel blocker and in this way limiting the passive entry of calcium into the chloride cells (Flik, 1990). Freshwater as well as seawater fishes have access to a continuous supply of calcium, and their main problem seems to be the limitation of calcium entry unless the water calcium levels are very low. This contrasts with terrestrial animals, which are fully dependent on the food as their — often limited — calcium supply. This

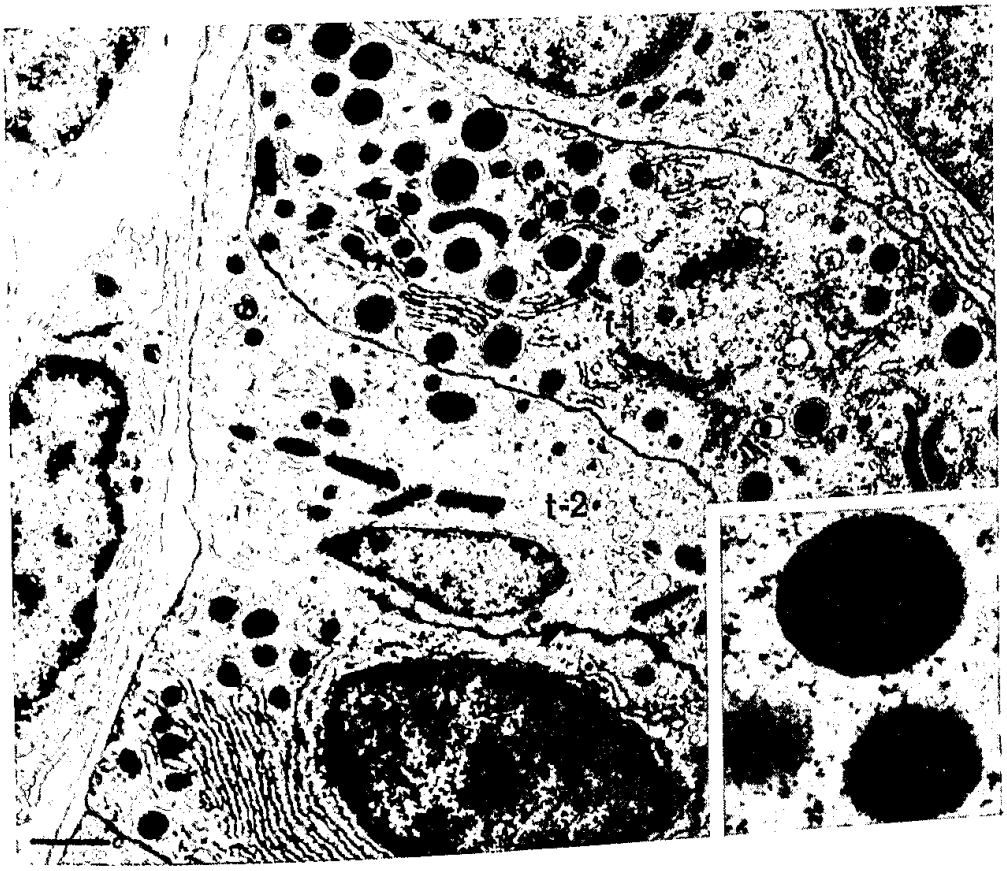

FIGURE 5. Electron micrograph of Stannius corpuscle of goldfish, showing type 1 cells (t-1), producing stanniocalcin, and type 2 cells (t-2) of unknown function; inset, secretory granules showing immunoreactivity to trout stanniocalcin (immunogold labeling); bar represents 1 μm.

difference between the aquatic and terrestrial conditions explains why the holostean and teleostean fishes depend for the control of their extracellular calcium on the hypocalcemic action of stanniocalcin. Contrastingly, in the terrestrial animals the endocrine factor dominating the control of extracellular calcium level is parathyroid hormone (PTH), which is a fast-acting and very potent hypercalcemic hormone. Parathyroid glands do not occur in fishes, and probably have evolved during the water-to-land transition of the early vertebrates (Wendelaar Bonga and Pang, 1991). Apparently, a PTH-like hypercalcemic hormone is not necessary in fishes, whereas terrestrial vertebrates do not require a potent hypocalcemic hormone like stanniocalcin (STC). One important question remains to be answered, and this is how the chondrichthyans and all other fishes that have no corpuscles of Stannius are able to maintain their plasma calcium levels, in particular in seawater.

Although true parathyroid glands are absent in fishes, immunoreactive PTH-, PTH-related peptide-, and STC-like activities were detected in coho salmon pituitary gland. Cytochemically, PTH- and STC-like immunoreactive material was localized in the neurohypophysis and preoptic area of platyfish brain. The authors suggested that this might reflect the presence of novel hypophysiotropic neuroendocrine systems (Fraser et al., 1991).

Vitamin D_3

The livers of marine teleosts are well known as rich stores of vitamin D_3 and have been applied for human use to prevent and treat rickets. Nevertheless, the function of vitamin D_3 and its metabolites in fishes is unclear. In the higher vertebrates, $1,25(OH)_2D_3$ is the most active metabolite that stimulates intestinal calcium uptake, bone remodeling, and calcium reabsorption in the kidney. Vitamin D_3 hydroxylation has been demonstrated in teleosts and lungfishes. Teleosts are able to produce $1,25(OH)_2D_3$ as well as $25(OH)D_3$ and $24,25(OH)_2D_3$, and high-affinity binding proteins for these metabolites have been demonstrated in the blood plasma in a few species. In blood plasma of lungfish and chondrichthyans, the presence of $25(OH)D_3$ has been demonstrated. Vitamin D_3 as well as $1,25(OH)_2D_3$ increased active intestinal calcium uptake in eels *in vivo* and *in vitro*. These substances were inactive when tested in perfused Atlantic cod, whereas $25(OH)D_3$ stimulated, and $24,25(OH)_2D_3$ decreased active calcium uptake in this preparation. In eel and cod $1,25(OH)_2D_3$ were hypercalcemic, whereas the other metabolites had no effect (Fenwick et al., 1984; Sundell and Björnsson, 1990; Sundell et al., 1993). Thus, although many aspects of the function of vitamin D_3 metabolites are still unclear, now there is biochemical and physiological evidence that the vitamin D_3 system is operating in fishes.

Pancreatic Hormones

In most vertebrates the pancreas contains islets of endocrine tissue among the trypsin-producing exocrine gland cells. The major hormones produced in most vertebrates are insulin, glucagon, pancreatic polypeptide, somatostatin, and pancreastatin. The physiological significance of the association of the endocrine and exocrine pancreatic tissue is unclear and does not seem to be essential. The analysis of the development of the islet tissue in fishes has contributed substantially to this notion. The distribution of the islet tissue in fishes is extremely variable. In the cephalochordate *Amphioxus*, which is missing a true pancreas, the immunoreactivity to insulin, glucagon, somatostatin, and pancreastatin is restricted to the open-type endocrine gut cells (see next section). The same situation is found in the ammocete larvae of lampreys, but in addition some insulin cells show an islet-like organization outside the gut epithelium. In adult agnathans, where the exocrine pancreas is represented by secretory cells in the intestinal epithelium, small islets are found in a ring-like association around the bile duct, as in hagfishes, or as a few relatively large islets, partly associated with the liver, in lampreys. In the gnathostomes, the islet cells evolved as a cell mass that mostly becomes associated with exocrine pancreatic tissue and gives rise to many small islets (e.g., in chondrichthyans), a few large islets (in most teleosts), or even a single large islet, such as in the swordtail fish. In many actinopterygian fishes the islet tissue is associated with some exocrine pancreatic tissue, forming large Brockmann bodies. In Polypteridae and Chondrostei, the pancreatic tissue with the islets is partially

included in the liver, while in some teleosts, islet tissue is associated with the spleen. Thus, the structural organization of the islet tissue is very variable in fishes and an association with the exocrine pancreatic tissue is not always present (Epple and Brinn, 1987).

In the islets of agnathans, immunoreactivity to insulin and two somatostatins has been demonstrated. No immunoreactivity to glucagon, glucagon-like peptide, or pancreatic polypeptide could be found. However, pancreatic polypeptide is a member of the family of neuropeptide-like peptides, and in lampreys immunoreactivity to several members of this family (neuropeptide Y, peptide XY, and anglerfish peptide Y) has been demonstrated. The reactive peptides were located in the same cells (Cheung et al., 1991). In the islets of gnathostomes immunoreactivity to insulin, glucagon, glucagon-like peptide, somatostatins, and pancreatic polypeptide was found (Epple and Brinn, 1987; Conlon et al., 1988). The specificity of the pancreatic polypeptide immunoreactivity has been doubted and, like agnathans, it probably is due mainly to peptide Y and related peptides of the neuropeptide Y family (Blomqvist et al., 1992). In rainbow trout and coho salmon glucagon and glucagon-like peptide — the products of a single gene coding for the preproglucagon sequence — are colocalized in one cell type (Nozaki et al., 1988). Another member of the glucagon family, oxyntomodulin, has been identified in the islet tissue of a gar pike (Pollock et al., 1988). Immunoreactivity to pancreastatin, a recently identified pancreatic hormone of mammals that inhibits glucose-induced insulin release, has been demonstrated in the islets of a hagfish, a ray, a holocephalian, and a teleost (Reinecke et al., 1991).

The functions of the islet hormones in fishes are incompletely known. The available evidence indicates that these are comparable to some extent with those in mammals. In the agnathans, removal of the islet tissue was shown to increase, and exogenous insulin to reduce, blood sugar levels, but the reports are not always consistent. However, isletectomy in the lamprey, *Geotria australis*, the only known vertebrate in which it is possible to remove all the pancreatic islet tissue without damaging other organs, was followed by a sharp rise in plasma glucose. The main source of the elevated blood glucose is unclear. Whereas in most fishes and the higher vertebrates the liver forms the main target for insulin, the small liver of lampreys forms an exception. The main energy store of these animals, the muscles of the body wall, are also rather insensitive to insulin (Epple et al., 1992). Insulin has been shown to be hypoglycemic in a number of gnathostome fishes. However, removal of the islet tissue by pancreatectomy is, in contrast to mammals where this operation is fatal, not followed by a dramatic hyperglycemia. The absence of this effect has been ascribed to the presence of insulin cells in the gut (in the chondrichthyans) or to the simultaneous removal of insulin and glucagon cells. At least in eels the hypoglycemic action of insulin is more balanced by the hyperglycemic action of glucagon than in mammals. Insulin stimulates glucose uptake by muscle and liver and promotes gluconeogenesis and lipogenesis in

these organs. An important function of insulin, at least in teleosts, is the stimulation of amino acid incorporation in tissue proteins, and amino acids are potent activators of insulin secretion (Epple and Brinn, 1987).

The understanding of the function of other islet hormones is even less than that of insulin. Glucagon causes hepatic glycogenolysis and lipolysis. It has been shown further to promote ion extrusion by the chloride cells in the gills of seawater teleosts. The first data on glucagon-like peptide show that it also is a potent stimulator of glycogenolysis. It has further gluconeogenetic and lipolytic activity. It seems to be more potent than glucagon. In fishes, in contrast to land vertebrates, it does not seem to have clear stimulatory effects on the secretion of other islet hormones such as insulin (Plisetskaya et al., 1989; Mommsen and Moon, 1989).

Somatostatins inhibit the release of insulin, glucagon, and pancreastatin in mammals, and there are indications that this holds for at least insulin and glucagon in teleosts. They further induce lipolysis and hyperglycemia (Sheridan et al., 1987). Hepatic glycogenolysis could be induced by somatostatin-14 and -25 in trout liver *in vitro* (Eilertson et al., 1991), indicating that the hyperglycemic effect of the hormone is direct, and not mediated by the inhibition of insulin secretion. Somatostatin has been shown to inhibit the ion secretion by chloride cells of seawater teleosts. The functions of the neuropeptide Y-like and pancreastatin-like factors in fishes are not known.

Gastro-Intestinal Hormones

The gastrointestinal tract of the vertebrates is an important source of hormones that control many important processes related to food digestion and energy metabolism. The hormones are produced by cells widely distributed among the normal epithelial cells of the gut. Although these endocrine cells have originally been proposed to originate from the neural crest, they are now considered to be true endodermal cells. They seem to have the capacity to perceive signals from the gut lumen and/or from the blood, and to respond to these signals by the release of hormones. Two structural types of secretory cells are distinguished: the open and closed types (Figure 6). Both types are in contact with the basal lamina. The open-type cells are also in contact with the gut lumen and are considered chemosensory cells. The closed cells have lost contact with the lumen and only seem to be able to transduce signals from the serosal side of the epithelium. The endocrine products of both types are released at the lateral and basal cell membranes (Vigna, 1986).

The hormones may diffuse to the blood circulation or they may influence neighboring cells by local diffusion (paracrine regulation). These neighboring cells may include other endocrine cells. For instance, in the mammalian stomach somatostatin secretion inhibits the release of gastrin. Both hormones are produced by separate cell types located in the epithelium of the stomach. To our knowledge paracrine effects have yet to be demonstrated in fishes. The neuropeptides produced by the gastrointestinal cells include the major hor-

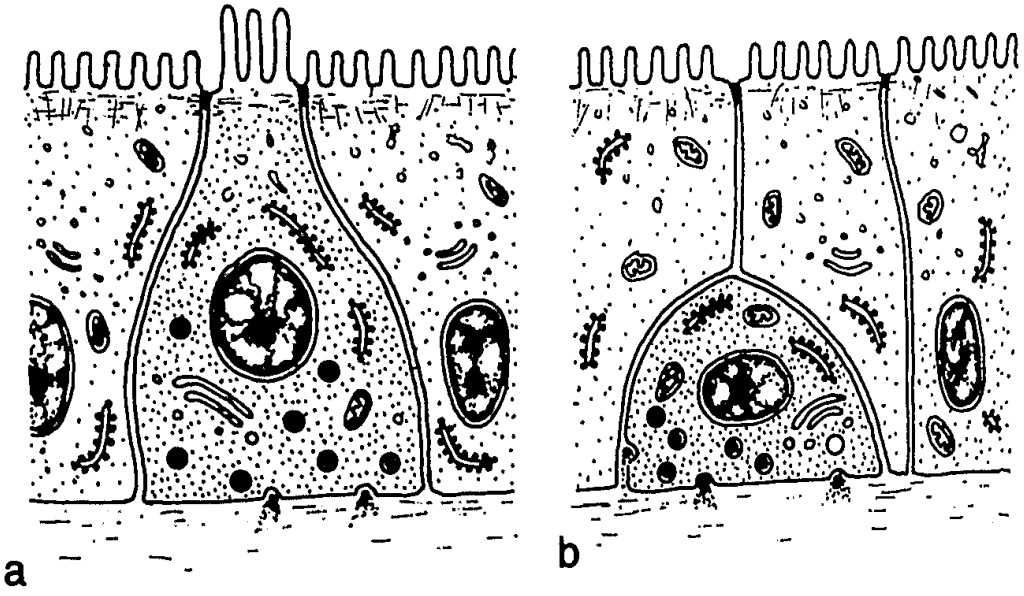

FIGURE 6. Gut endocrine cells of the open type (a) and of the closed type (b). Cells of both types are in contact with the basal lamina; hormonal messengers are released by exocytosis of secretory granules; the open-type endocrine cells extend microvilli-like processes, probably with a receptor function, into the gut lumen; cells of the closed type are lacking this specialization.

mones of the pancreatic islets: glucagon, glucagon-like peptide, somatostatin, pancreatic polypeptide, and, in agnathans and chondrichthyans, insulin. Recently, pancreastatin has also been demonstrated (Nozaki et al., 1988; Reinecke et al., 1991). This circumstance, together with the common embryological origin of the gastrointestinal and the pancreatic endocrine cells, has initiated the concept of the gastro-entero-pancreatic (GEP) endocrine system, which includes the endocrine cells of the gut and the islets. Although this name is less appropriate for fishes because of the absence in several fish species of a close association of the islets with the pancreatic tissue, the underlying idea has derived much support from comparative and developmental studies in fishes (Epple and Brinn, 1987; see also above). Immunoreactivity to many other types of gastrointestinal neuropeptides of mammals have also been demonstrated in fishes. These include gastrin/cholecystokinin, bombesin, enkephalin, vasointestinal peptide, tachykinins, neuropeptide Y-like peptides (in particular peptide YY, Blomqvist et al., 1992), neurotensin, secretin, gastric inhibitory peptide, and serotonin (Bjenning and Holmgren, 1988; Burckhardt-Holm and Holmgren, 1989). This list is certainly incomplete. Several of the mammalian gut hormones have not been studied extensively, and undoubtedly other hormones still await identification.

The study of the function of the gut hormones is hampered by the circumstance that the classical endocrine approach for function analysis (extirpation and replacement therapy) is not feasible for the endocrine system of the gut because of its diffuse nature. It has been suggested that the endocrine function of these cells is important for the adjustment of the release of digestive

secretions to the amount and composition of the nutrients in the digestive tract. Some peptides may stimulate (gastrin/cholecystokinin, bombesin, substance P, serotonin) or inhibit (vasointestinal peptide) gut motility, or have excitatory effects on gastric epithelia (enkephalin, neurotensin) and increase gastric acid secretion (bombesin), but experimental evidence is limited (Bjenning and Holmgren, 1988).

Neuropeptides of the Gills

In addition to the gastrointestinal system, other organs may contain dispersed neuroendocrine cells. Such cells, producing bioamines and peptides frequently located in the same cells, are particularly common in the respiratory tract of terrestrial vertebrates (Scheuermann, 1987). A local, paracrine function has been ascribed to these cells, although a more hormonal action cannot be excluded. Such cells have also been described in pulmonary structures in fishes. For instance, serotonin-producing cells occur in the pulmonary epithelium of lungfish and in the swim bladder of the air-breathing *Polypterus* (Zaccone et al., 1992). Serotonin-producing cells have been described in the gills of chondrichthyans, actinopterygians (Figure 7), and the African lungfish (Laurent, 1984; Zaccone et al., 1993). Some of the open-type cells exhibited immunoreactivity for metenkephalin and leu-enkephalin. These cells responded by increased release of their secretory material to environmental hypoxia and reduction of the water calcium concentration. Leu-enkephalin-positive cells were further found in the gills of a lamprey. We have shown neuropeptide Y immunoreactivity in closed-type cells in teleost gill (Figure 7). It has been suggested that these cells modulate gill functions and that they may act as chemoreceptor cells (Bailly et al., 1992).

Angiotensin

Angiotensin II is the main product of the renin-angiotensin system, in fishes as well as in the higher vertebrates. It originates from a precursor molecule (angiotensinogen) that is processed by renin, produced mainly in the kidneys by the juxtaglomerular cells, to angiotensin I, and by converting enzyme, produced in several organs, to angiotensin II.

A renin-angiotensin system has been found in all major groups of fishes, with the exception of the agnathans (Sokabe and Ogawa, 1974). Until recently it was also thought to be absent in chondrichthyans, but now there is good evidence for its presence and functioning in elasmobranchs. Similar to other fishes, juxtaglomerular cells showing renin-like immunoreactivity could be demonstrated in the kidneys of several elasmobranchs (Lacy and Reale, 1989) and renin-like and angiotensin I converting enzyme-like activities were found in a dogfish. Both activities were present in the kidneys, whereas converting enzyme-like activity was further found in gills, spleen, and brain (Uva et al., 1992).

In mammals, angiotensin II has effects on blood pressure, aldosterone secretion, and drinking behavior. In fishes, angiotensin II increases systemic

FIGURE 7. Light micrographs of immunoreactive neuroendocrine cells in the gills of fishes; PAP method; bars represent 20 μm. (a) Cluster of met-5-enkephalin-positive neuroendocrine cells in the filamental epithelium of brown trout; (b) neuropeptide Y-positive neuroendocrine cells in the filamental epithelium of the teleost *Oreochromis mossambicus* (G. Geeraedts, A. Coenen, and S.E. Wendelaar Bonga, unpublished); (c) serotonin-positive neuroendocrine cells in the basal area of the filamental epithelium of the catfish *Ictalurus nebulosus* (a and c from Zaccone et al., *Acta Zool.*, 73, 177, 1992. With permission from Pergamon Press, Oxford, UK).

blood pressure, an effect that is indirect and mediated by catecholamines (Nishimura, 1987). In an elasmobranch, administration of angiotensin II stimulated the secretion of 1α-hydroxycorticosterone (Hazon and Henderson, 1985), and in teleosts that of cortisol (Perrott and Balment, 1990). Angiotensin II further initiates drinking, particularly in seawater teleost fishes (Hazon et al., 1989). Adaptation of euryhaline teleosts to increased water salinity requires renal antidiuresis effected by reduction of the glomerular filtration rate. This can be effected by angiotensin II, and this may therefore be an essential hormone for adaptation to seawater (Brown et al., 1990). Recovery from pharmacologically induced hypotension in flounders could be inhibited by

injection of captopril, a converting enzyme inhibitor, and this also reduced plasma cortisol levels. This indicates a physiological function for endogenous angiotensin II in body fluid volume control and interrenal steroidogenesis (Perrott and Balment, 1990).

Melatonin

In fishes, the pineal organ (epiphysis) receives and integrates light stimuli, and this input is transformed into electrical and hormonal responses. In this respect the pineal organ of fishes is comparable with that of most amphibians and reptiles, and contrasts with that of birds and mammals. In the latter groups the receptor function of the pineal organ is present only during early development. Subsequently the pineal organ reduces to a gland mainly consisting of neuroendocrine transducer cells that receive information about the light regimen via the eyes.

The pineal organ projects from the roof of the diencephalon. In most fishes the organ forms a pineal complex with another projection of the diencephalic root, the parapineal organ. In teleosts it is rudimentary, and most adult teleosts only have a pineal organ. It has a lumen that in most fishes is in open connection, via the pineal stalk, with the third ventricle. The tissue of the pineal wall consists of pinealocytes, glial cells, and neurons. The pinealocytes of fishes are true photoreceptor cells with a lamellated outer segment that contains the photopigments and that is comparable with the retinal cones of the eyes. The outer segments project into the pineal lumen and are connected, via an inner segment that is rich in mitochondria, with the cell body that contains the nucleus. The cell body forms the secretory part of the cell. It may produce neurotransmitters as well as hormonal messengers. One or more cytoplasmic processes from these cells may be synaptically connected with other cells. The skin may be less pigmentated and the skull less calcified in the area overlying the pineal. This pineal window facilitates the illumination of the organ.

The major hormone of the pineal organ is melatonin, an indole amine. In the higher vertebrates several other potential messengers have been identified, including peptides. Melatonin-like material has been demonstrated in the cell bodies of the pineal photoreceptor cells of fishes and is most likely secreted from the basal parts of these cells. In this respect these cells are comparable to the photoreceptor cells of the retina, that also have the capacity to secrete melatonin. At least in the higher vertebrates most of the melatonin present in the circulation originates from the pineal organ, and this seems also applicable to fishes. Retinal melatonin may have a paracrine function (Falcón and Collin, 1991). Different types of photoreceptor cells may be present in the pineal organ, and not all of these seem to secrete melatonin (Tamotsu et al., 1990).

The pineal plays a crucial role in adaptation to the light regime. The secretion of melatonin — predominantly in the dark phase — mediates the adjustment of behavioral and physiological processes to the daily light-dark

cycle and the annual cycle in the daily light period. Virtually all body processes are influenced, from mitotic activity to locomotory activity (including migration), skin pigmentation, or the timing of reproduction. In fishes, the pineal organ functions as an endogenous oscillator, showing a circadian rhythmicity — also *in vitro* (Iigo et al., 1991) — which is adjusted via the photic input of the pineal. In the retina of pike the melatonin content showed a peak during the dark phase, similar to the pineal gland (Falcón and Collin, 1991). The functional relationship between melatonin production in the retina and in the pineal in fishes is unknown. Van Eys and Wendelaar Bonga (1981) have shown that, for the control of skin pigmentation in a teleost, the photic input of both the eyes and the pineal organ is essential.

CONCLUSIONS

The above survey shows that our knowledge of the neuroendocrine system of fishes is expanding rapidly. The system shares its general pattern with that of all other vertebrates. The developments in the endocrine research follow those in mammals. More and more messenger molecules are identified, and it appears that these messengers are not only produced in the brain and the classical endocrine system but also in many other organs. The same messenger molecule, or closely related variants, may be produced in different organs in the same organism and, depending on the location, function as neurotransmitter, neuromodulator, hormone, or paracrine messenger.

These conclusions are based on a limited set of data, particularly when it is taken into account that the fishes are a very diverse group of animals, and, with over 20,000 representatives, comprise more than half of the vertebrate species. As far as the identification of the messengers is concerned, originally this has been almost exclusively based on immunoreactivity to antisera raised against mammalian hormones and on mammalian assay systems. This implies that hormones unique for fish may have escaped attention. This may, furthermore, have caused an overestimation of the similarities between the fish and mammalian hormones. The structural analysis of more and more fish hormones has shown that this indeed has taken place and that the fish equivalents of mammalian hormones often show significant structural differences not only between fishes and higher vertebrates, but also between various groups of fishes, between species of the same systematic category, or between different production sites in the same species. This structural diversity is further expressed in the production of different variants of one hormone by the same cell type, for instance in the case of prolactin, somatostatin, growth hormone, or MSH. The diversity is already immense, even though the neuroendocrine system of only a small number of fishes has been explored.

For most species this exploration has been superficial, and restricted to the accumulation of data on the location of immunoreactivity to one or more

hormone antisera. More detailed analysis of the structure of the messengers and analysis of their function is badly needed for many of the known fish hormones. This also holds for the receptors of these hormones, an important aspect of neuroendocrine communication that has been neglected in this chapter. It should be kept in mind that hormone receptors have been evolved in parallel with the hormones concerned, and therefore the receptors may be expected to show a similar structural diversity as the hormones.

Although the neuroendocrine system of fishes conforms to the general pattern of the vertebrates, there are some interesting differences that are more fundamental than the many structural variants of the messenger molecules or the structural organization of the neuroendocrine tissues. First, these concern both the function of hormones that are present in fishes as well as the terrestrial vertebrates, and, second, the presence of hormones that are typical for fishes. Not surprisingly, most aspects that are unique for fishes are related, directly or indirectly, to their aquatic habitat. Fishes have a very close interaction with the ambient water. As mentioned above, there is an intensive exchange of monovalent and divalent ions, related to physiological functions such as water and ion regulation, respiration, acid-base balance, and excretion of waste products. The physiological importance and complexity of these exchanges are reflected by the large number of hormones that are implicated in the control of these substances. Prolactin, growth hormone, cortisol, glucagon, and somatostatin are only a few examples of hormones with important ionic regulatory functions and effects in fishes and not, or to a limited extent, in the terrestrial vertebrates. Messengers with ionic regulatory capacities that are absent, at least as true hormones, in the terrestrial vertebrates are the urotensins and stanniocalcin. Somatolactin and melanophore concentrating hormone probably also belong to this category. Some other endocrine messengers found in mammals have not been demonstrated in fishes. This may be caused by lack of research efforts or specific detection techniques rather than the absence of fish equivalents of these hormones. However, there is good evidence for the absence of one hormone, PTH, and the near-absence of another, aldosterone. The appearance of PTH in the land vertebrates has been connected with the change during the water-to-land transition of the vertebrates, which implied the loss of water as a readily accessible calcium source. The absence of aldosterone in most fishes (traces of this corticosteroid have been found in some, mainly nonteleostean species; aldosterone may have physiological significance only in lungfishes) is more difficult to understand, because it is the mineralocorticoid of the higher vertebrates. This function is exerted by cortisol or, possibly, corticosterone in fishes, which are also the main glucocorticoids in these animals. Perhaps the regulation of energy metabolism and the control of ion regulation are so closely linked in purely aquatic animals that these functions can be combined easily in one hormone.

This chapter shows that fish endocrinologists have contributed substantially to our knowledge of the evolution of the vertebrate neuroendocrine system.

Their work is also valuable for improving fish farming, by increasing survival, reproduction, and growth rate of fish. These applied aspects of fish endocrinology will certainly become more and more important in the near future.

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